NASAL MUCUS AND INFLUENZA VIRUSES

II. A NEW TEST FOR THE PRESUMPTIVE DIAGNOSIS OF INFLUENZA INFECTION

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The biological properties of nasal mucus, as learned in the preceding study, are such that by appropriate methods the effect of viral action can be detected by the examination of a single sample (Fazekas de St Groth, 1952). The characteristic which changes during enzymic degradation was termed 'inhibitor index', and a simple technique has been devised for its determination. As it could be shown that even minute amounts of influenza viruses were capable of inducing significant changes in the inhibitor index values on interaction *in vitro*, the test was expected to be adequate for the detection of human influenza infection, since nasal secretions contain sufficient amounts of virus during the acute phase of the disease.

The validity of this proposition could be submitted to experimental testing during the 1950 epidemic of influenza A-prime in Melbourne. The corresponding epidemiological data are contained in the paper by Anderson, French & Kalra (1952); the present communication is based on the same material, and should be regarded as a complementary investigation examining the merits of a new diagnostic method, without adding anything to the epidemiological information already available.

EXPERIMENTS

All *materials and methods* were the same as in the preliminary study, and have been fully described in the accompanying paper (Fazekas de St Groth, 1952).

General technique of experiments

Nasal swabs were taken from patients with clinical influenza, or from persons in contact with such cases, by the attending physician or a member of the Epidemiological Unit of the Hall Institute. The swabs were kept in serially numbered sterile test-tubes, so that the patient's name and the clinical diagnosis were unknown at testing, and when replicate or consecutive daily samples were taken from the same person, they carried different numbers. As a rule the swabs were tested on the day of arrival; approximately one-third of the samples was re-titrated on the following day. Throat washings and a sample of blood were also collected from most patients at the time when the first swab was taken.

Each swab was soaked in 2·0 ml. of normal saline for about an hour at room temperature. At the end of this period 0·25 ml. of the extract was made up to 1·50 ml. with saline (1 : 6); this common starting dilution was distributed into

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three tubes, and from these three sets of parallel doubling dilutions were made in 0·25 ml. volumes of normal saline. The use of nine steps (dilutions from 1 : 6 to 1 : 1536) was found sufficient to cover the range of titres encountered. A standard drop containing exactly five agglutinating doses of WSE-i was added to each tube of the first row, MEL-i to the second, and LEE-i to the third. After pre-incubation for 30 min. at room temperature 0·025 ml. (one standard drop) of 5% fowl red cells was added to every tube, the racks were thoroughly shaken and left standing for another 30 min. The readings, made by the pattern of settled cells, gave the inhibitory titres of each sample against the three indicators. From these titres the inhibitor index was calculated by the formula

$$\frac{10 \times \text{MEL-titre} + \text{LEE-titre}}{\text{WSE-titre}},$$

as described previously.

As the results of virus isolation became available (3–8 days after determination of the corresponding inhibitor indices), the code numbers were replaced by the patients' names, and the comparison of findings begun. The final correlation tests were made only 3–4 weeks later, after the serological investigations had been completed.

Changes in the inhibitor index during natural influenza infection

To avoid extensive repetition, it is proposed to present grouped averages rather than individual results. Preceding this, however, a representative set of findings will be given in detail, viz. that obtained on the first ten cases to come under observation and followed for at least 5 days. All members of this random group were young adults, students of a large public school in Melbourne. Patients XI and XII belong to the same group but were 24th and 28th respectively in the chronological order. They are included in Table 1 because of the special interest their case holds.

The first swabs were taken on 5, 6 or 7 July 1950 (11 and 13 July respectively for patients XI and XII), as a rule within 24 hr. of the patient's reporting ill at the school infirmary. In the tables this time will be denoted 'day 1'. It should be realized, however, that it refers to the first day of testing rather than to the onset of illness, the latter being somewhat uncertain because of the personal factor involved.

At least a fortnight after the first swab, usually between days 16 and 20, a second bleed was taken, and also a specimen of nasal mucus ('convalescent sample'). The inhibitor indices, together with the results of the orthodox tests for influenza, are tabulated below.

Table 1 contains examples of most types of response encountered in this study. Thus, there are clearly negative findings (patients V and VII) in which the inhibitor index remained normal throughout, and neither could virus be isolated nor rise in specific antibody detected. Patient VIII, on the other hand, represents a typical case of influenza both by clinical and laboratory criteria, yet the inhibitor index values did not deviate from the normal during the acute stage of illness.

The rest of the patients, all of whom reacted positively in the accepted objective tests for influenza, showed abnormally high inhibitor indices during the acute stage of their disease. Yet, the extent and duration of these changes is far from uniform. In some instances (e.g. III and VI) the inhibitor index was only slightly outside the normal range, and only for a short period; others (e.g. I and IV) showed extremely high values, but reverted to normal after 3 days; a third group (e.g. II, IX and X)

Table 1. *The changes in nasal mucus during clinical influenza*

	Diagnostic tests													
				Inhibitor index on day										
Patient	Virus isolation	Anti-haemag-glutinin	Comple-ment fixing antibody	1	2	3	4	5	6	7	8	9	10	11	Convales-cent sample*
I	+	+	+	**100**	**193**	**71**	28	25	34	.	.	.	.	.	34
II	+	+	+	**97**	35	**73**	42	**67**	**68**	35	28	.	.	.	32
III	+	+	+	**54**	36	41	28	.	.	.	.	.	.	.	32
IV	+	+	+	**103**	**111**	48	**72**	32	41	.	.	.	.	.	38
V	−	−	−	38	36	29	27	33	24	.	.	.	.	.	31
VI	+	+	+	**54**	40	36	27	38	.	.	.	.	.	.	31
VII	−	−	−	26	27	27	27	36	33	25	.	.	.	.	30
VIII	+	+	−	31	33	38	22	41	.	.	.	.	.	.	40
IX	+	+	+	48	**63**	**75**	.	**63**	**69**	**71**	.	.	.	.	29
X	+	+	+	**66**	**54**	48	48	**83**	**99**	.	**74**	**52**	50	.	20
XI	−	+	+	29	32	23	45	**62**	80	**55**	**87**	.	.	.	33
XII	−	+	+	40	25	37	46	32	45	**106**	**72**	**65**	**92**	**43**	21

Inhibitor indices significantly different from the normal are set in heavy type.
* 'Convalescent sample', taken between days 14 and 22.

had a moderately distorted inhibitory pattern persisting for at least 6 days. Another important feature of the results, which will be discussed fully at the end of the paper, is the finding of extensive daily variations in the inhibitor index, such as were not seen in normal persons. This was evident in all tests, and is most conspicuous where normal and abnormal patterns alternate (e.g. II, IV and X).

Patients XI and XII belong to a special group. For the first 4 or 6 days respectively their nasal secretions were of the normal inhibitory pattern, and then abruptly changed to abnormal. The fact that no virus could be isolated on day 1, but significant antibody rises were recorded on days 17 and 22 respectively, tends to support the contention that at the time of admission these patients were free from influenza, and acquired the disease while in hospital. Among the thirty-eight patients who have been followed for more than 7 days there were five cases of this type. Throat washings of three were tested by amniotic inoculation, and were found negative; isolation of virus was not attempted from the other two.

Because of the small numbers involved Table 1, of course, does not give a true indication of the frequency with which the various types of response occurred; indeed, certain combinations do not appear in it at all. Thus the false positive reaction (distorted inhibitory pattern with negative findings both in virus isolation and antibody titrations) and some instances where two of the tests were positive

and two negative, are not represented. However, all of these will appear in the contingency tables below. Based on a sufficient number of observations, these will give a statistically valid measure of the distribution of different types of response.

One characteristic feature of the changes in the nasal inhibitor reveals itself quite clearly even in this small sample. In the stage of convalescence the inhibitor index returns to values within the normal range. Only on a single occasion was a value of 52 read on the 18th day. This patient showed abnormal inhibitory patterns on days 2 and 4 of his illness, but reverted to normal between days 5 and 7. As his case represents less than 1% of the observations, and the 'convalescent sample' is only very slightly outside the normal limit, it is considered an extreme variant rather than an exception to the rule.

Comparison of changes in the inhibitor index with the outcome of laboratory tests for influenza

For the sake of concise presentation changes in the inhibitor index will be expressed in an abbreviated form. Reactions which exceed the normal average by less than twice its standard deviation are considered normal. Thus any inhibitor index below the value of 50 will be labelled 'negative'. Slight increases, between 51 and 65, will be designated as $\pm$. The range between 66 and 80 is $+$, and all values above 80 are $++$.

As it is of some interest to know whether a single test gives as much information as a series of daily tests, in the tables which follow the results obtained by the orthodox diagnostic methods are compared with the inhibitor index as found (*a*) on the 1st day only, (*b*) on the 1st and 2nd days of testing, and (*c*) on more than 2 days. The values published refer always to the highest reading observed. For purposes of the χ^2-tests all inhibitor indices exceeding 50 are considered positive. This condensation of the contingency tables is both legitimate because of the clearly isotropic distribution, and advisable in view of the small numbers in certain classes. The latter was also accounted for by applying Yates's correction for continuity, throughout.

Besides the square contingency (χ^2), computed on the null hypothesis of independence between the results of any two techniques, it was felt desirable to give also a quantitative measure of association. The coefficient of contingency, the analogue of the coefficient of correlation for continuous functions, served this purpose. The original formula for $t \times s$ tables (Tschuprow, 1925),

$$T^2 = (\chi^2/N)/\sqrt{[(s-1)\,(t-1)]},$$

is simplified in the case of 2×2 tables to $T = \sqrt{(\chi^2/N)}$, where T is the coefficient of contingency and N the number of observations on which χ^2 was calculated. The values of T vary between 0 (no correlation) and 1 (absolute correlation).

It is quite evident from Table 2 that patients from whom virus could be isolated tend to have abnormal inhibitor indices, while the majority of those with negative throat washings exhibit no change in their nasal inhibitor. The high χ^2-values indicate that such findings would be extremely improbable ($P < 0{\cdot}001$) were the two reactions independent.

The comparison of contingency coefficients discloses a rather remarkable trend: tests on the nasal mucus on 2 successive days slightly improve the agreement between the two methods, but the correlation becomes definitely worse if based on a larger series of observations. The late appearance of abnormal inhibitor index values (cf. patients XI and XII in Table 1) accounts for this anomaly since, at least formally, this group has to be regarded as 'false positives'. The fact that repeated tests give more accurate information becomes clear when the steady decline in false negatives is considered ($10/74 > 5/61 > 3/51$).

Table 2. *Correlation between diagnostic tests for influenza*

(Inhibitor index and virus isolation.)

	Amniotic isolation of virus					
	(*a*)		(*b*)		(*c*)	
Inhibitor index*	+	−	+	−	+	−
+ +(>80)	5	0	7	0	10	4
+(66–80)	3	1	5	1	5	1
±(51–65)	10	3	6	5	5	3
−(<50)	10	42	5	32	3	20
	$\chi^2 = 23{\cdot}46$		$\chi^2 = 20{\cdot}88$		$\chi^2 = 15{\cdot}11$	
	$T = 0{\cdot}563$		$T = 0{\cdot}585$		$T = 0{\cdot}544$	

* The highest inhibitor index, as found (*a*) on the 1st day, (*b*) on the 1st and 2nd days, (*c*) on more than 2 days.

All inhibitor indices exceeding 50 are considered positive in the statistical tests.

In the case of virus isolation it is of prime importance to obtain the throat washing in the acute phase of the disease. Failure to do so could have caused some of the discrepancies seen in Table 2. This source of error is not present in tests for antibody, provided sufficient time is given for the development of immunity. An interval of 2–3 weeks, as adhered to in the present study, is generally considered adequate. A limitation of another kind however is inherent in serological tests based on the comparison of two titres, namely the definition of the smallest increase which can be considered significant. In this respect the antihaemagglutinin test presents greater difficulties than the evaluation of a rise in complement-fixing antibodies. Normal sera, as a rule, show some inhibitory activity, and the level of these non-specific reactions varies with the strain of virus used in the test. The current epidemic strains were characterized by a rather high level of non-specific inhibition. Consequently, a fourfold rise in titre was probably a too exacting criterion, rendering the test less sensitive than usual.

Again, as when compared with the isolation of virus from throat washings, the changes in the inhibitor index are closely correlated with the trend of the anti-haemagglutinin titres (Table 3). Indeed, both the χ^2-values and the contingency coefficients attain levels incompatible with independence between the two tests.

Among the patients who showed distorted inhibitory pattern but less than fourfold antihaemagglutinin rise, there were several whose antibody titres were twice as high during convalescence as in the acute phase of the disease. From many of these virus has been isolated or a significant rise in complement-fixing antibody

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obtained. Though it is reasonable to assume that the actual rise in antihaemagglutinin would have been more than fourfold but was masked by the initially high non-specific inhibitory titre, the cases are grouped among the 'false positives' in respect to the inhibitor index test.

Table 3. *Correlation between diagnostic tests for influenza*

(Inhibitor index and rise in antihaemagglutinin.)

	Rise in serum antihaemagglutinin					
	(*a*)		(*b*)		(*c*)	
Inhibitor index	+	−	+	−	+	−
+ +(>80)	5	0	6	0	11	1
+(66–80)	3	1	5	1	5	1
±(51–56)	7	2	6	4	4	4
−(<50)	8	52	6	47	3	42
	$\chi^2=33{\cdot}02$		$\chi^2=28{\cdot}77$		$\chi^2=34{\cdot}09$	
	$T=0{\cdot}640$		$T=0{\cdot}619$		$T=0{\cdot}693$	

As non-specific reactions do not interfere in the complement-fixation test using the small (30 S) antigen of influenza, any positive reading was regarded as diagnostic of recent infection. Accordingly, the instances in which the two tests disagree rest in all probability on genuine failures of one or the other, and cannot be blamed on systematic or technical errors.

Table 4. *Correlation between diagnostic tests for influenza*

(Inhibitor index and rise in complement-fixing antibody.)

	Rise in complement-fixing antibody					
	(*a*)		(*b*)		(*c*)	
Inhibitor index	+	−	+	−	+	−
+ +(>80)	5	0	7	0	11	1
+(66–80)	4	2	5	3	4	2
±(51–65)	11	0	9	1	6	1
−(<50)	7	54	6	50	3	44
	$\chi^2=42{\cdot}93$		$\chi^2=38{\cdot}54$		$\chi^2=40{\cdot}82$	
	$T=0{\cdot}719$		$T=0{\cdot}690$		$T=0{\cdot}753$	

The area of agreement between the two tests is extensive (Table 4), especially if the results of repeated determinations of the inhibitor index are compared with the outcome of the complement-fixation tests. This increase in correlation when more than two determinations are compared with the results of the serological tests, as is evident between the columns (*b*) and (*c*) of Tables 3 and 4, rests at least partly on the same phenomenon which caused the lowering of the contingency coefficient in Table 2, namely on the late appearance of distorted inhibitory patterns in a small group of patients.

Behaviour of the nasal inhibitor of normal persons during the epidemic period

Partly preceding, partly in parallel with the investigation of material coming through the Epidemiological Unit, nasal swabs were taken from members of the

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Institute's staff free from clinical signs of influenza and showing no serological evidence of recent infection at the end of the epidemic period.

In all, 137 samples from twenty-nine normal persons have been tested. Of these, 134 gave an inhibitor index of less than 50, i.e. would have been regarded as negative by the criterion defined above. Outside the negative range there were two readings of 53 and one of 56; these would have been given the score of ± (doubtful positives). The arithmetic mean of the whole group works out to 33·29 with a standard deviation of ± 7·83, the distribution being insignificantly different from normal when the inhibitor index values are plotted on an arithmetic scale. As has been pointed out already in the preceding paper, both the mean and its variance are indistinguishable from those established from a group of normal donors in an inter-epidemic period. This agreement also shows that the normal inhibitor index is free from significant seasonal variation.

Effect of diverse respiratory diseases on the inhibitor index

Though it has been shown in the previous sections that the changes in the biological properties of nasal mucus are closely linked with objective signs of influenza infection, and are of an order that could not be expected with the observed frequency to occur by normal variation, the possibility has not been formally excluded that the rise in inhibitor values was a non-specific phenomenon arising in various, unrelated pathological conditions of the respiratory organs. Titrations were made therefore on a large group of hospitalized patients whose clinical diagnosis was not influenza. It was not deemed necessary to show that these patients in fact did not have influenza, partly because the tests were done 6 months after the epidemic, and partly because their inhibitor index values, as will be seen from Table 5, did not differ from the normal.

Table 5. *The inhibitor index during respiratory diseases*

Clinical diagnosis	Inhibitor index
Common cold	29, 47, 31, 39, 33, 35, 30, 39, 50, 44, 43, 38, 23, 19, 32, 25, 35, 35, 40, 19, 31, 39, 36, 27, 30, 22, 29, 36, 27
Pharyngitis	31, 31, 33, 25, 36, 43
Tracheo-laryngitis	41, 27, 34, 27, 30, 46, 33
Bronchitis	34, 44, 38, 28
URTI(?)	22, 35, 36, 31, 29
Bronchiectasis	39, 43, 27, 42, 44, 34, 24, 32, 31, 34, 24, 28, 39
Emphysema, chronic	34, 28, 26
Asthma	30, 27, 26, 20, 41, 35
Lobar pneumonia	34, 38, 29, 46, 30, 18, 45, 27, 36, 36
Broncho-pneumonia	21, 33, 29, 39, 29, 27, 27, 34, 49, 32, 30, 27, 40, 30, 31, 40, 45, 19, 29
Virus pneumonia	29, 36, 22, 31, 45, 41, 29, 25
Tuberculosis pulm.	32, 27, 22, 41, 26, 20, 36, 29, 38, 41, 39
Carcinoma laryng.	33, 40
Carcinoma pulm.	39, 40, 27
Pleuritis sicca	39, 31, 39, 27
Pleuritis serosa	21, 46, 29
P.U.O.(?)	30, 28, 36, 43, 29, 40, 25

Nasal swabs were collected and tested by the standard method; Table 5 gives the results in detail. It is evident that a representative group of acute or chronic diseases of diverse etiology and localization did not have any effect on the inhibitory properties of nasal secretions.

DISCUSSION

The evidence presented above is compatible with the hypothesis that in the acute phase of influenza infection there is enough virus in the upper parts of the respiratory tract to cause measurable degradation of nasal mucus. This fact can be used for the recognition of infection, as it has been shown that the enzyme is an integral part of the infective particle (Gottschalk & Perry, 1951), and there is a simple and sensitive technique available by which minimal action of the virus can be detected.

Ease of performance, however, is not sufficient recommendation for a diagnostic test. Before its acceptance can be proposed, it has to be shown that it is reproducible, specific and sensitive. The experimental part of this study has been conducted with these points in mind, and the material is large enough to give statistically valid answers to all the three questions. Indeed, as far as reproducibility is concerned, it has already been shown in the preliminary experiments, (Fazekas de St Groth, 1952) that the technique of measurement is adequate in so far as its variance is smaller than the natural variation of inhibitory titres in nasal secretions, and that the findings can be expressed in a form both concise and informative, viz. the 'inhibitor index'. This has the average value of 33 for normal human nasal mucus, and it could be shown that less than 5 % of the normal values were higher than 50. This limit has been set, arbitrarily, as the borderline between normal and abnormal values.

The specificity of the method was assessed by comparison with the results of accepted laboratory tests for influenza. The relevant observations, contained in Tables 2–4, show good though not complete correlation. Whether compared with the isolation of virus or with the appearance of specific antibodies, the majority of pairs tend to agree; there is, however, an important fraction of both false negative and false positive readings. The former is of less importance, since it does no more than lower the sensitivity of the method. False positive reactions, on the other hand, are misleading and introduce a systematic error. Whether a reaction labelled 'false positive' is in fact false, cannot be decided with certainty at present as none of the orthodox tests is known to be absolutely sensitive, i.e. capable of detecting every case of influenza infection. To reduce the inherent uncertainty when examining this question more fully, it is proposed to pool the information and regard a case as positive if it has reacted positively in *any* of the three accepted tests. Treated in this fashion the results show that amniotic isolation of virus detected influenza in 27/38 cases, and there was no instance in which positive isolation would not have been followed by the appearance of at least one type of antibody. The antihaemagglutinin test agreed with other tests in 22/27 cases, and there was one instance in which only this test gave a positive reading. The appearance of complement-fixing antibody coincided with other positive tests in 26/30 cases, and disagreed

in 1 case. Abnormal inhibitor index was observed 32/39 times in agreement with other tests, and there were five false positives. It should be realized that this method of evaluation is slightly biased against the test on nasal mucus, since of the 44 positive cases there were only 21 in which all the other tests have been performed; the rest were tested by one or two methods only.

It would appear, then, that the inhibitor index is increased in roughly 11 % of the cases without concomitant objective evidence of influenza infection. Yet, it is difficult to believe that this is a chance occurrence as some of the observed values were exceptionally high (>100). An interpretation, which unfortunately could not be verified during the epidemic because the discrepancy was noted too late, is to suppose that in some cases the virus multiplies only in the lining of the nasal cavity, without reaching the lower parts of the respiratory tract. Such limited growth is known to occur in mice (Nakanishi, 1950), probably also in ferrets (unpublished observations), and under special experimental conditions in humans (Henle, Henle, Stokes & Maris, 1946). In this situation the virus could not be cultivated from throat washings, and should not reach high enough concentrations to provoke a marked rise in antibody levels. Whether this speculation has any relation to reality could be decided, during an epidemic, by attempting virus isolation from the nasal secretions of the appropriate patient, which should be positive; and testing the pattern of bronchial mucus, which should be normal. Until the point in question has been settled it is safer to regard the nasal mucus test as giving one false result for every nine positive readings; the hope may be entertained however that, owing to our ignorance, this estimate is too high.

The degree of sensitivity of the methods can be judged from the information on which specificity was evaluated. Thus, the order of increasing sensitivity is: virus isolation (27/38, or 71 %), rise in antihaemagglutinin (23/28, or 82 %), abnormally high inhibitor index (32/39, or 82 %), and presence of complement fixing antibodies (27/31, or 87 %). The percentages are, of course, only approximations, but they should be of the right order of magnitude. The comparison shows that changes in the inhibitor index are about as sensitive as the other tests. It is also evident that none has 100 % sensitivity, and therefore they should be used in conjunction for any more exacting work.

The sensitivity of the new method can be discussed also in more general terms. Distortion of the inhibitory pattern of any mucoid depends on the relative amounts of inhibitor and virus present, on the time of interaction, and on the intrinsic enzyme action of the virus, as measured by its position in the inhibitor gradient (Stone, 1949 *a*, *b*). This latter will vary with different strains of virus, and there is no way of forecasting reliably the behaviour of future epidemic strains. It is known, however, from experiments on twelve human influenza strains that between 0·1 and 1 agglutinating dose of virus is sufficient to cause a significant rise in the inhibitor index in an hour at 37° C. (Fazekas de St Groth, 1952). To appreciate this point fully, it is preferable to express the concentrations of virus in terms of infectivity rather than of haemagglutinating power. Thus, it can be shown that for all egg-adapted strains 1 haemagglutinating unit contains $10^{5\cdot4}$ infective particles per 0·05 ml. ($= 10^{5\cdot56}$ ID_{50}); the same result is obtained in mouse infectivity

tests, provided the intercept of the probit dose-response curve with the abscissa is taken as the end-point, and not an arbitrary degree of lung consolidation (Fazekas de St Groth, to be published). The infectivity of throat washings from acute cases of human influenza varies between 10^2 and 10^5 per 0·05 ml., i.e. $10^{4·6}$–$10^{7·6}$ per 20 ml. of throat washing (French, E. L., 1950; personal communication). From the few cases where nasal swabs have been tested for infectivity, the values seem to be only slightly lower than in the corresponding throat washings. Supposing 24 hr. contact with the nasal mucus, it follows that as small amounts as 10^3 ID_{50} of virus could bring about significant changes in the inhibitor index.

The conditions under which the viral enzyme has to act in the living organism are almost certainly more complex than considered in the above calculation. A pointer in this direction is the fact that normal and abnormal inhibitor indices may alternate on consecutive days (cf. Table 1). This phenomenon may be caused either by the intermittent flow of virus from the infected tissues, or by the appearance of freshly secreted mucus which, having a normal pattern, could mask a smaller amount of degraded inhibitor. No attempt has been made to decide between the alternatives, though the question might be of some importance inasmuch as it determines whether consecutive daily tests would give more information than a single test. From the tables it would appear that not much is gained by increasing the number of titrations, as the elimination of missed positives is just about made up for by the appearance of false positives. The obvious exception to this rule is the group of patients who contracted influenza at a later stage of their sojourn in hospital. It is possible, however, that the frequency with which such cases occur might have been lower had the bulk of the material not come from a school infirmary.

To conclude, estimation of the inhibitor index in nasal secretions can be regarded as an objective test for influenza virus infection. It is definitely inferior in specificity to the accepted laboratory tests, but has the great advantage that it can be applied in the acute phase of the disease, is easy and inexpensive to perform, and gives an answer within an hour's time. Thus it possesses the attributes of a presumptive rather than a definitive test. Its main scope lies in the screening of larger groups, and in deciding from which patients the isolation of virus should be attempted; also, it might be found useful in the following up of individual cases. To whatever purpose the test be put, it should always be regarded as an additional and not as an alternative technique—as we have no perfect diagnostic method for influenza at present, the only way accurate information can be gained is by balancing and controlling the imperfections of one test by the parallel use of others.

SUMMARY

Changes in the inhibitory properties of human nasal mucus were found to be positively correlated with objective signs of influenza virus infection during the 1950 epidemic. In approximately 80 % of the cases the inhibitor index was significantly increased during the acute stage of the disease, and reverted to normal during convalescence.

Various respiratory diseases other than epidemic influenza did not cause similar changes.

Examination of the inhibitory pattern of nasal mucus is proposed as a presumptive test for influenza virus infection, and its merits are discussed under the headings of reproducibility, specificity and sensitivity.

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